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Milk as source of bioactive compounds

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› Questions I was asked to answer

› What is state of the art within the field in question?

- › Huge areas of research
- › Very active field
- › Many individual topics
- › Many new findings

› What are the hypotheses?

- › Enhancement of the general knowledge on the importance of milk for health and nutritional status
- › Production of milk with designated health effects
- › Use of milk components as functional foods
- › Use of milk components as pharmaceuticals

› Which results have been achieved?

› What does future work focus on?

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Overview literature

- **Bioactive components in milk**
  - Edited by Z. Böze –
  - Springer, 2008
- **Bioactive components in Milk and Dairy products**
  - Edited by Y.W. Park
  - Wiley, 2009
- **Technological and Health Aspects of Bioactive Components of Milk**,
  - Edited by Hannu Korhonen
  - Volume 16, Issue 11, 2006 Add to my Quick Links

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Overview of biological components

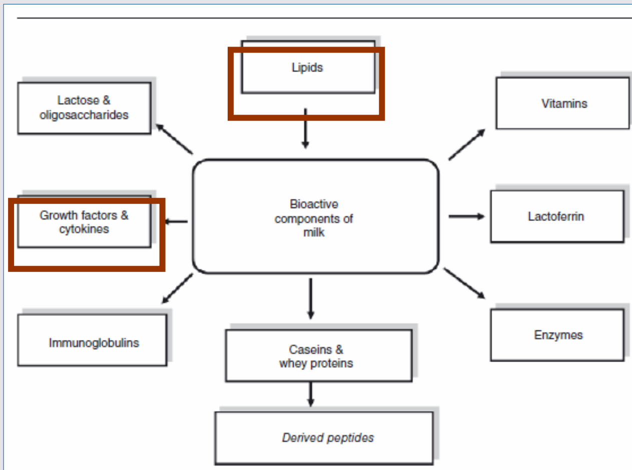

Figure 1.1. Schematic representation of major bioactive functional compounds derived from milk.

Park, 2009, In: Bioactive components in Milk and Dairy products

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Major milk proteins –

Table 2.1. Major bioactive protein components of bovine colostrum and milk: Concentration, molecular weight, and potential biological functions*

Protein	Concentration (g/L) Colostrum	Concentration (g/L) Milk	Molecular Weight Daltons	Biological Activity
Caseins (α_{s1}, α_{s2}, β, and κ)	26	28	14.000–22.000	Ion carrier (Ca, PO_4, Fe, Zn, Cu), precursor for bioactive peptides immunomodulatory, anticarcinogenic
β-lactoglobulin	8.0	3.3	18.400	Vitamin carrier, potential antioxidant, precursor for bioactive peptides, fatty acid binding
α-lactalbumin	3.0	1.2	14.200	Effector of lactose synthesis in mammary gland, calcium carrier, immunomodulatory, precursor for bioactive peptides, potentially anticarcinogenic
Immunoglobulins	20–150	0.5–1.0	150.000–1000.000	Specific immune protection through antibodies and complement system, potential precursor for bioactive peptides
Glycomacro-peptide	2.5	1.2	8.000	Antimicrobial, antithrombotic, prebiotic, gastric hormone regulator

Korhonen, 2009, In: Bioactive components in Milk and Dairy products

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Lactoferrin	1.5	0.1	80.000	Antimicrobial, antioxidative, anticarcinogenic, anti-inflammatory, iron transport, cell growth regulation, precursor for bioactive peptides, immunomodulatory, stimulation of osteoblast proliferation
Lactoperoxidase	0.02	0.03	78.000	Antimicrobial, synergistic effects with immunoglobulins, lactoferrin, and lysozyme
Lysozyme	0.0004	0.0004	14.000	Antimicrobial, synergistic effects with immunoglobulins, lactoferrin, and lactoperoxidase
Serum albumin	1.3	0.3	66.300	Precursor for bioactive peptides
Milk basic protein	N.A	N.A	10.000–17.000	Stimulation of osteoblast proliferation and suppression of bone resorption
Growth factors	50μg–40 mg/L	<1 μg–2 mg/L	6.400–30.000	Stimulation of cell growth, intestinal cell protection and repair, regulation of immune system

*Compiled from Pihlanto and Korhonen (2003) and Korhonen and Pihlanto (2007b); N.A. = not announced.

Korhonen, 2009, In: Bioactive components in Milk and Dairy products

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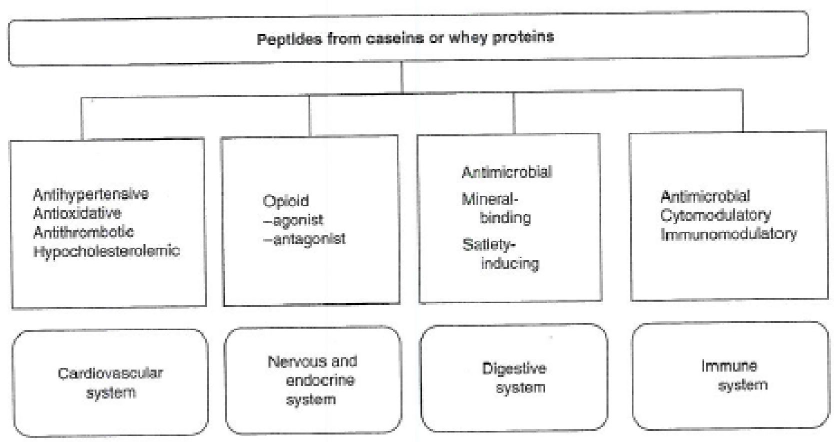

Figure 2.2. Physiological functionality of milk-derived bioactive peptides.

Korhonen, 2009, In: Bioactive components in Milk and Dairy products

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Milk lipids

› Bovine milk contain more than 400 fatty acids

Important milk fat components

› Saturated fatty acids
› Unsaturated fatty acids
› Short and medium chain fatty acids
› Omega-3 fatty acids/omega-6 fatty acids
› CLA – Conjugated linoleic acid
› Trans fatty acids
› Cholesterol
› Phospholipids
› Sphingolipids

Biological functions

› Part of organs and cell membranes
› Body energy storage
› Involved in regulation of many biological processes

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Research on bioactive components in milk

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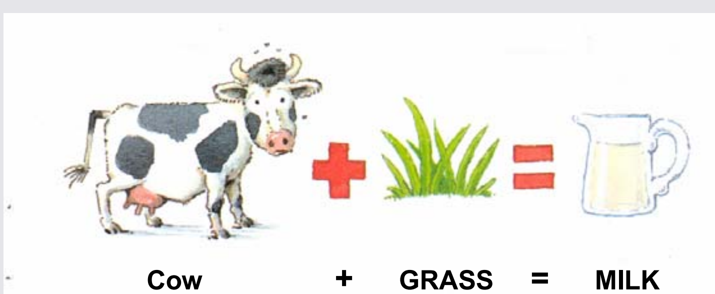
Large variation in milk composition from variation in the feed



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Most fatty acids from feed are C18 fatty acids
- Fatty acids composition varies between feeds

Long chain fatty acids in different feeds

	Fatty acid, g/100 g FA				
	C16:0	C18:0	C18:1	C18:2	C18:3
Soybeans[1]	12	4	23	53	6
Rapeseed cake[1]	6	2	59	22	9
Sunflower seeds[1]	6	4	26	63	0.3
Peanut oil[2]	12	3	51	30	-
Linseed[2]	7	4	23	15	51

[1] Nielsen et al. (unpublished)
[2] Kelly et al. (1998)

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Milk fat composition - mol %

Fatty Acid	Cow
Short Chain	
C4:0	11
C6:0	5
C8:0	1
Medium Chain	
C10:0	3
C12:0	10
C14:0	3
Long Chain	
C16:0	23
C18:0	10
C18:1	29
C18:2	2
C18:3	<1

Synthesized **de novo**
- All saturated

Common fatty acids
– originate **from feed**:

C16:0 - Palmitic acid - palmitinsyre
C18:0 - SterARic acid - stearinsyre
C18:1 - Oleic acid - oliesyre
C18:2 - Linoleic acid - linolsyre
C18:3 - Linolenic acid- linolensyre

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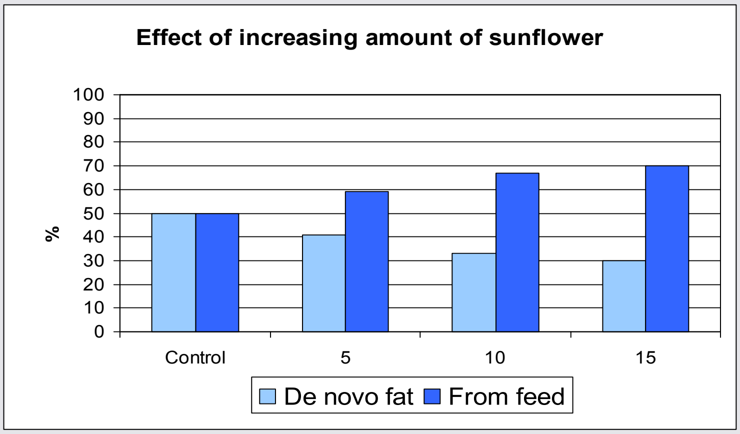
Relative amount of de novo and feed fatty acids in milk
- Up to 75 % of fat in milk can come directly from feed



Sejrsen et al.

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Factors affecting milk fat composition

Feed
- Amount of fat
- Fat source
- Rouhage type

Season

Cow factors
- Breed
- Stage of lactation
- Lactation number
- Genotype

→

Saturated/usaturated fat

Short vs. Medium chain

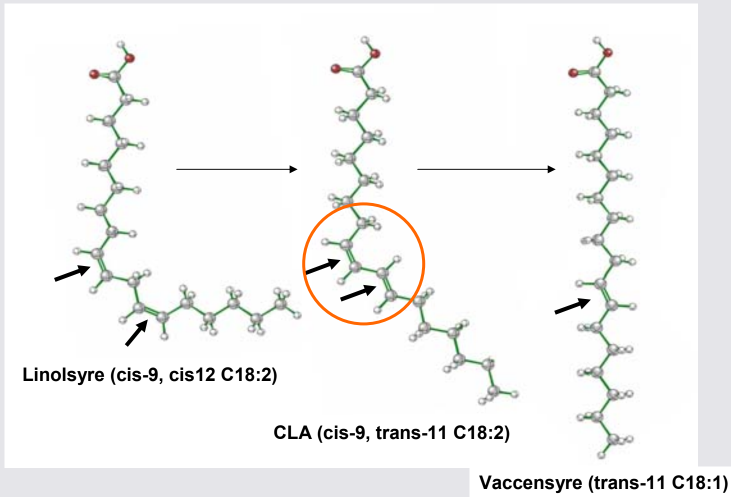
Conjugated linoleic acid (CLA)

Omega 3/omega 6-fatty acids

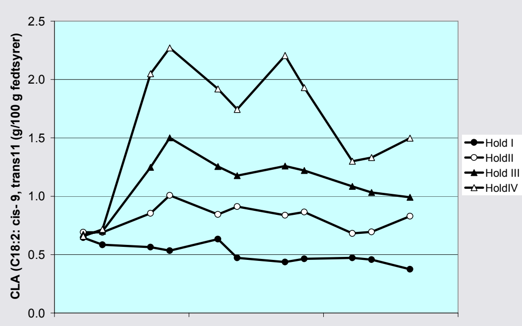
Trans fatty acids
- trans 10 vs. trans 11

Fat soluble vitamins
- vitamin E and D

CLA - many positive health effects shown i lab animals
- anticancer, antidiabetic, anti-artherogenic
- antiobesity, immunstimulating



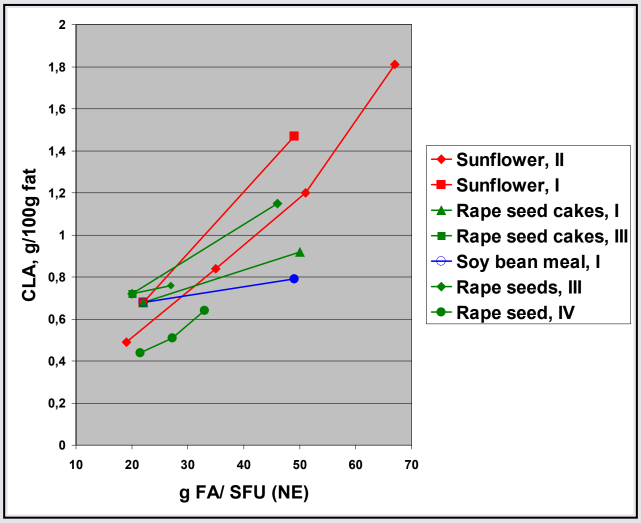
Influence of amount of fat in the diet on milk composition



Increasing amount of sunflower

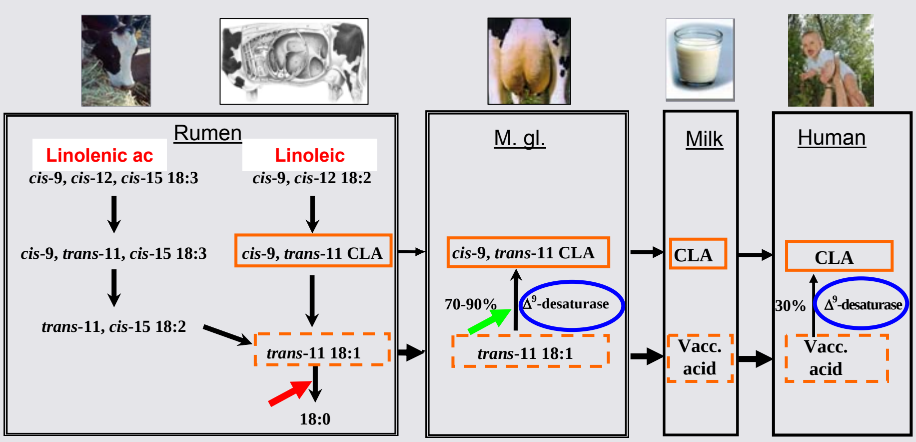
Nielsen et at,

Summary of results on effect of fat source and amount of fat in the diet

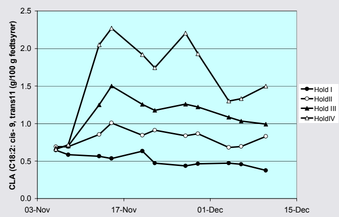


Nielsen et al.

CLA - route from feed to human tissues - upgraded in the mammary gland



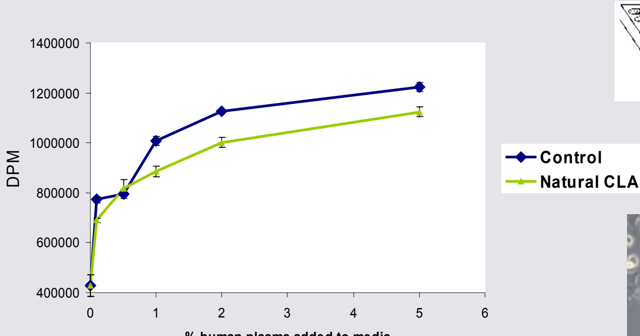
Human intervention trial
- Tine Tholstrup et al

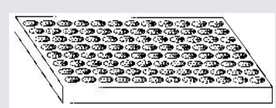


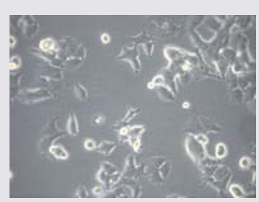
Results on fatty acid incorporation and different risk factors published Tholstrup et al.

Human intervention study

Cell proliferation in vitro of breast cancer cells (MCF7) tend to be reduced by plasma from human subjects receiving







Nielsen et al., 2005

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Effect on milk on short chain fatty acids

Treatment	C4:0	C6:0	C8:0
Grass silage	3.59	1.97	1.17
Corn silage	2.69***	1.36***	0.87***
Sunflower	4.32	12.2	28.5
Rape seed	1.92***	8.27***	18.9***

Sejrsen et al.

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Effect on milk on medium chain fatty acids

Exp.	Treatment	C12:0	C14:0	C16:0
2	Grass silage	2.64	9.79	21.8
	Corn silage	2.34*	8.59**	18.6***
3	Sunflower	4.32	12.2	28.5
	Rape seed	1.92***	8.27***	18.9***

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Effect on milk on Omega-3 fatty acids

Treatment	C18:3 n3	C20:3 n3
Grass silage	0.64	
Corn silage	0.41***	
Sunflower	0.14	0.08
Rape seed	0.44***	0.16***

Fresh pasture and fish oil are best sourses of omega-3

Sejrsen et al.

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Trans fatty acids in milk

- often changed together with other changes

Treatment	Total C18:1 trans fatty acid	C18:1 Trans 11	C18:1 Trans 10
Control diet	5.01	1.77	0.66
Corn silage[A]	9.69***	2.18	4.60***
Control diet	1.93	0.54	0.51
Rape seed[A]	9.25***	1.80***	5.11***

[A]High levels of unsaturated fat and starch in the diet

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Mammary Expression of Genes Involved in Lipid Metabolism is Affected by Dietary Supplementation with Unsaturated Fat

Jeppe Westh Møller, Troels Bjørn, Peter Theil, Martin Tang Sørensen, and Kristen Sejrsen[#]
[#]University of Aarhus, Dept. Animal Health and Bioscience, P.O. Box 50, 8830 Tjele, Denmark

Genes	Diet (% DM)			P
***De novo* fatty acid synthesis**	Control	5%	10%	ANOVA
ACC*	1.00	0.46	0.39	0.060
FAS	1.00^{a}	0.51^{b}	0.44^{b}	0.035
Fatty acid desaturation				
FADS1	1,00^{a}	0,70^{ab}	0,19^{b}	0,006
SCD	1.00^{a}	0.48^{ab}	0.41^{b}	0.046
Lysosomal cholesterol transport				
NPC1	1,00^{a}	0,49^{a}	0,19^{b}	<.0001
Sphingolipid synthesis				
SPTLC2	1.00^{a}	1,08^{a}	0,71^{b}	0,001
Transcriptional regulators				
PPARG	1,00^{a}	0,86^{ab}	0,47^{c}	0,012
SCAP (activator of SREBP-1)	1,00^{a}	0,90^{ab}	0,47^{b}	0,0312
SREBP-1	1.00^{a}	0.42^{b}	0.39^{b}	0.034

QPCR analysis
Genes analyzed without significant differences in gene expression: **ABCG2, FABP, FADS2, FAT, INSIG1, LASS2, LPL, SGMS2, SPTLC1, UGCG2.** We were unable to obtain usable detection of **ELOVL5 and ELOVL7.**

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Hormones and growth factors in milk

Milk contains many hormones and growth factor

-Biological role unclear

- -Suggestions
  - -Role in the mammary gland?
  - -Role in the development of the newborn?
  - -Excretion route?

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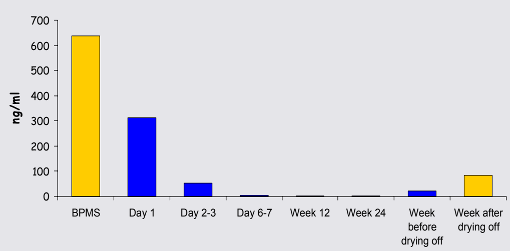
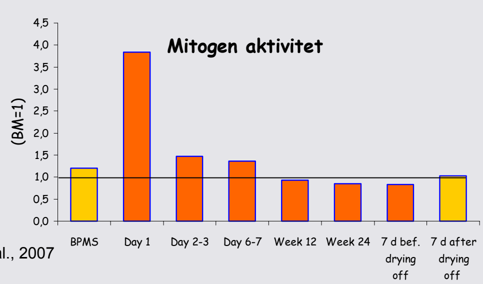
IGF-I





Sejrsen et al. 2000; Purup et al., 2007

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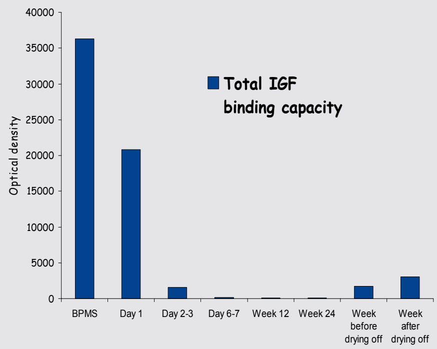

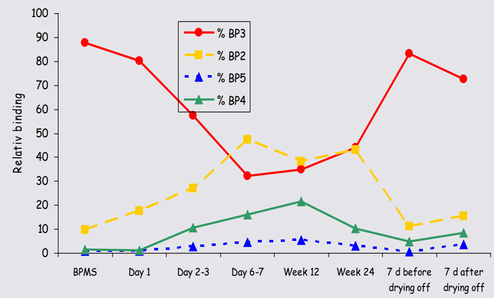

Sejrsen et al. 2000; Purup et al., 2007

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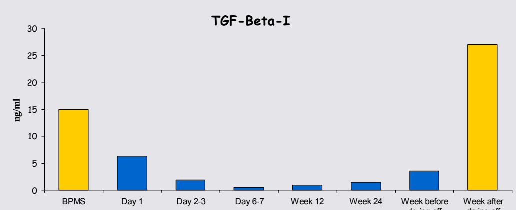

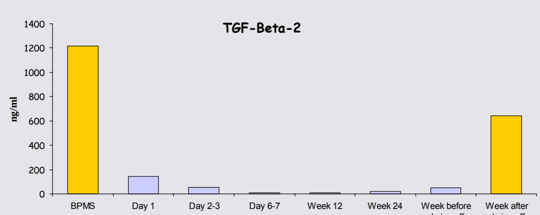

We have also measured growth hormone and insulin in milk

Purup et al., 2007

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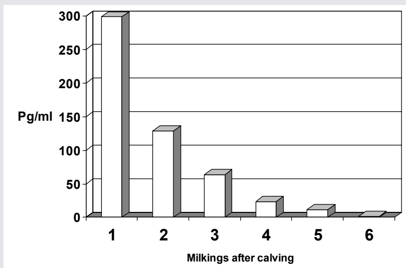
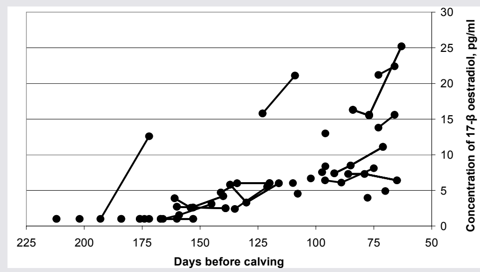
Concentrations of non-conjugated 17-β oestradiol (pg/ml) in skim milk postpartum (Andersen et al. 2007).





Andersen et al. 2008

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Factors tested in bioassay with mammary epithelial cells

Growth stimulating	Growth inhibiting	No direct effect on growth
IGF-I	IGFBP-2	Estradiol
Des(1-3)IGF-I	IGFBP-3	GH
IGF-II	TGF-β1 (high conc.)	Prolactin
Insulin	FGF-2 (high conc.)	Leptin (mouse and human)
EGF	Retinol	β-carotene ?
TGF-α	Retinal	Heparin
TGF-β1 (low conc.)	Retinoic Acid	
FGF-1	Retinol palmitate	
FGF-2 (low conc.)	Enterolactone	
Amphiregulin	Enterodiol	
FCS	Accel GF	
Heifer serum		
Mammary gland extract		

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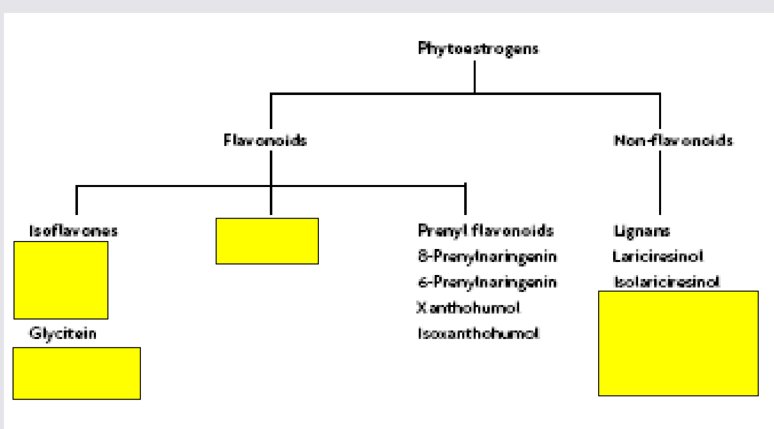
- **Uncertainties about the role of growth factors**
  - Variation in milk content?
  - Stability in the intestinal tract?
  - Are they absorbed?
  - Effects on the gut tissue development?
  - Effects on tissue repair after injury?
  - Interactions between different growth factors?
  - Interactions with other components -
    - proteases/fatty acids/lactoferrin/retinoids?
  - Still unidentified growth factors?

Phytoestrogens
- Milk content?
- Only few papers
- Relevance for human health?



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Concentration of phytooestrogens in the feedstuffs (mg/kg DM).

Feedstuff	Period	Secoiso-lariciresinol	Mateiresinol	Daidzein	Genistein	Coumesterol	Formononetin	Prunetin	Biochanin A
Alfalfa silage	1-2	9.07	<lod	0.64	1.18	7.98	14.60	<lod	<lod
	3-4	7.66	<lod	<lod	<lod	5.35	13.51	<lod	<lod
Clover grass silage	1-2	0.31	<lod	7.54	17.44	<lod	452.03	14.47	32.23
	3-4	4.24	<lod	9.80	26.27	<lod	371.51	6.53	65.87
Maize silage	1-2	<lod	<lod	0.77	1.06	<lod	9.67	<lod	<lod
	3-4	<lod	0.81	<lod	5.74	<lod	9.80	<lod	<lod
Rolled barley	1-2	<lod	<lod	1.30	1.57	<lod	6.01	<lod	<lod
	3-4	<lod	<lod	<lod	0.40	<lod	1.80	<lod	<lod
Rape seed cake	1-2	<lod	<lod	0.71	0.48	<lod	7.62	<lod	<lod
	3-4	<lod	<lod	0.59	<lod	<lod	6.74	<lod	<lod
Dried sugar beet pulp	1-2	<lod	<lod	4.60	2.88	<lod	16.89	<lod	<lod
	3-4	<lod	<lod	2.28	2.28	<lod	10.42	<lod	<lod

Phytoestrogens in cows milk (nmol/l) in relation to lucerne, maize and clover grass silage in the feed. (Andersen, Sejrsen, Hansen Møller & Weisbjerg, 2008)

	Experimental treatments[12]				
	Lucerne	2/3 lucerne +1/3 maize	1/3 lucerne +2/3 maize	Clover grass silage	SE
Isoflavones					
Formononetin	5.80^{a}	5.99^{a}	5.89^{a}	8.77^{b}	1.10
Daidzein	4.69^{a}	5.07^{a}	4.84^{a}	9.83^{b}	1.17
Equol	26.4^{a}	21.4^{a}	16.6^{a}	782.7^{b}	72
Biochanin-A	1.15^{a}	3.00^{b}	2.06^{ab}	2.77^{b}	1.29
Genistein	6.10^{abx}	6.53^{ab}	5.67^{a}	7.55^{bx}	1.44
Prunetin	3.50^{a}	4.85^{b}	4.47^{ab}	4.39^{ab}	1.19
Lignans					
Secoisolariciresinol	88.5	82.7	84.1	84.7	12.7
Matairesinol	1.76^{ab}	1.32^{ab}	2.22^{b}	0.49^{a}	1.37
Enterolactone	183^{a}	191^{a}	291^{b}	162^{a}	67
Enterodiol	0.66^{ab}	0.28^{a}	1.05^{b}	0.81^{ab}	0.62
Courmestans					
Coumestrol	9.21^{ay}	8.13^{a}	4.92^{ay}	0.33^{b}	4.12

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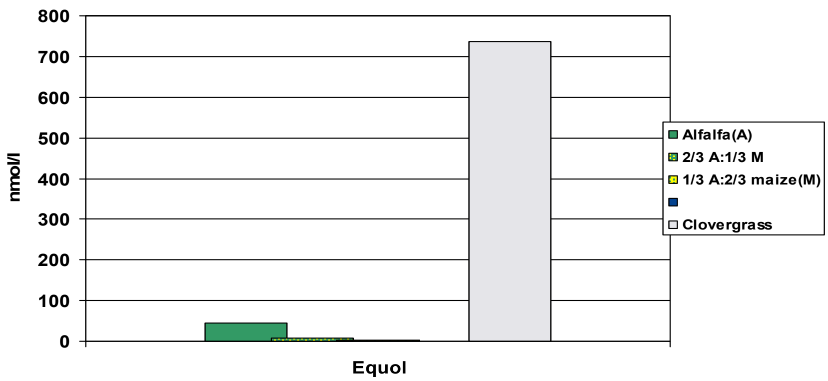
Isoflavones in milk
i relation to feed composition



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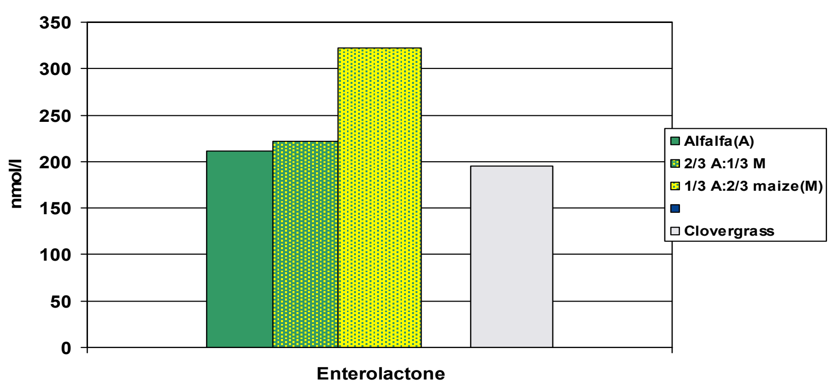
Lignans in milk
i relation to feed composition



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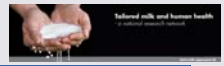
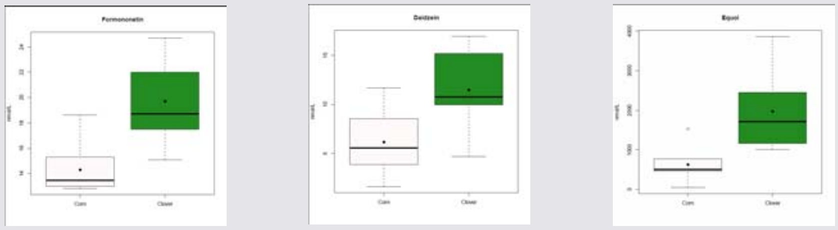
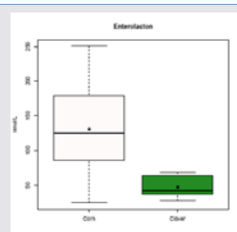


Clovergrass feeding increased the content in milk of: **isoflavenoids**



The content in milk of **enterolactone** is decreased

Sejrsen et al., unpublished



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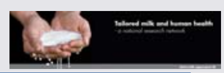
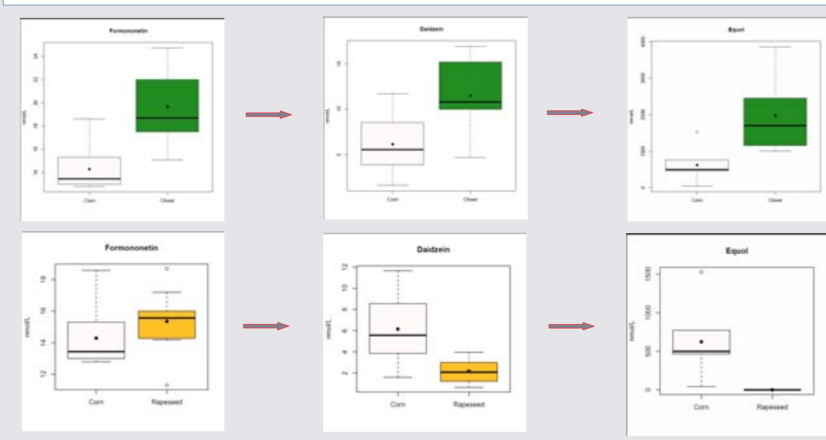
Sejrsen et al., unpublished



Equol is formed in the digestive tract from formonetin and daidzin



Feeding rape seed block formation of equol - this suggest that variation in diet may explain why a part of the human population does not form equol

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De enkelte projekter

1. **Milk with designated health-effects – production and evaluation**
   - Kristen Sejrsen, AU-DJF
2. **Cholesterol transport and milk-proteins**
   - Torben Ellebæk Petersen, AU-NAT
3. **Green feed improves the nutritional properties of the milk-fat fraction**
   - Lars Hellgren, DTU
4. **Milk protein, obesity and the metabolic syndrome**
   - Chr. Mølgaard, KU-Life
5. **Sund og velsmagende mælk baseret på græsmarks-afgrøder**
   - Jacob Holm Nielsen, AU-DJF
6. **Colostrum for gut protection and recovery**
   - Per Sangild, KU-Life